SUPPLEMENTAL INFORMATION

RNA remodeling activity of DEAD-box proteins tuned by protein concentration, RNA length and ATP

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SUPPLEMENTAL INFORMATION CONTAINS:

SUPPLEMENTAL FIGURE 1

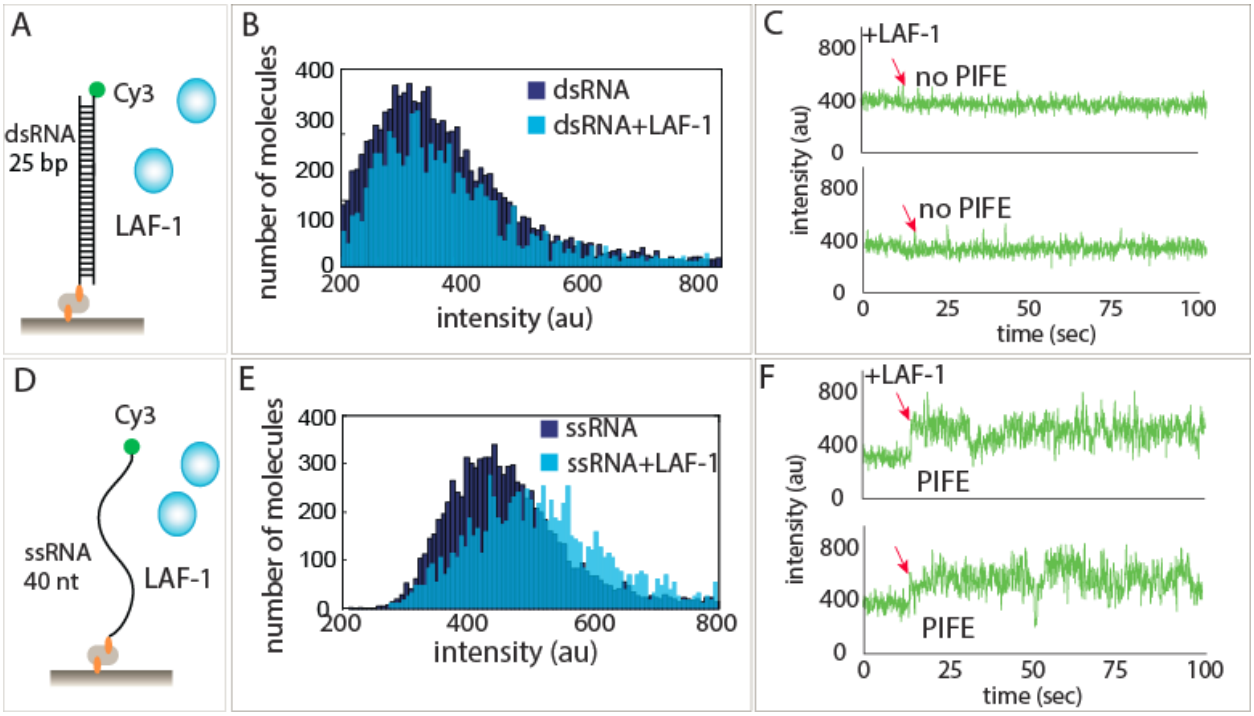

FIGURE S1 LAF-1 binds to ssRNA, not dsRNA, Related to Figure 1. (A, D) Cy3 labeled dsRNA and ssRNA substrate used for single molecule protein induced fluorescence enhancement (smPIFE) imaging. (B, E) Intensity histogram obtained for LAF-1 binding to dsRNA and ssRNA. The intensity shift is seen only in the case of ssRNA, suggesting LAF-1 binding to ssRNA, not to dsRNA. (C, F) Representative single molecule intensity traces for dsRNA and ssRNA. The intensity remains constant in dsRNA whereas it rises to higher level and fluctuates upon LAF-1 binding to ssRNA. Due to the fluctuating signal, the overall enhancement in intensity is not dramatic in the histogram shown in (E).

SUPPLEMENTAL FIGURE 2

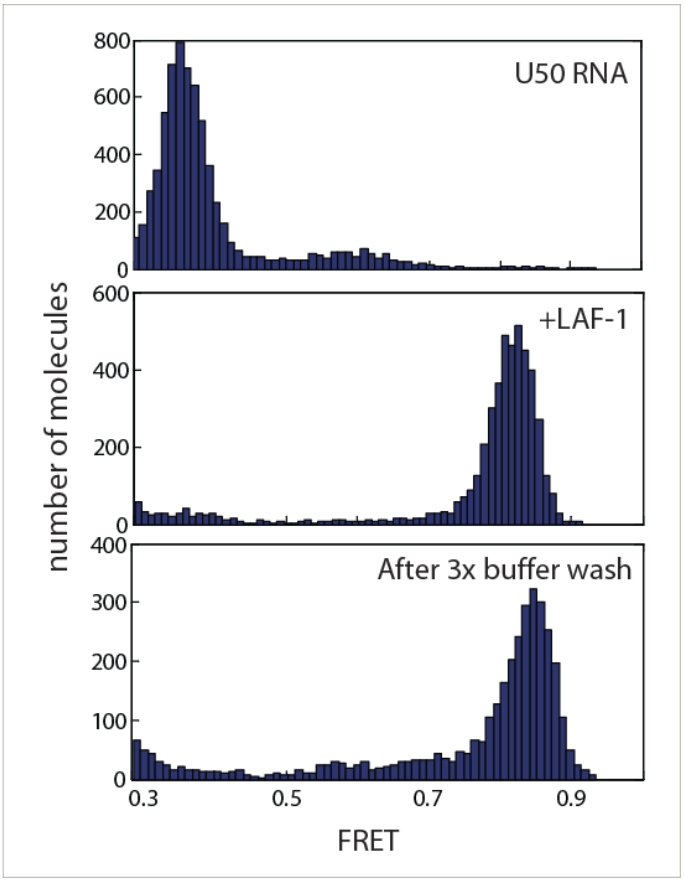

FIGURE S2 Buffer wash does not remove LAF-1 from RNA, Related to Figure 2. FRET histogram of U50 RNA (top), after addition of 10 nM LAF-1 (middle) and after three times of buffer wash (bottom). The high FRET induced by LAF-1 binding remains the same even after excessive buffer wash, indicating a highly stable binding of LAF-1 to ssRNA.

SUPPLEMENTAL FIGURE 3

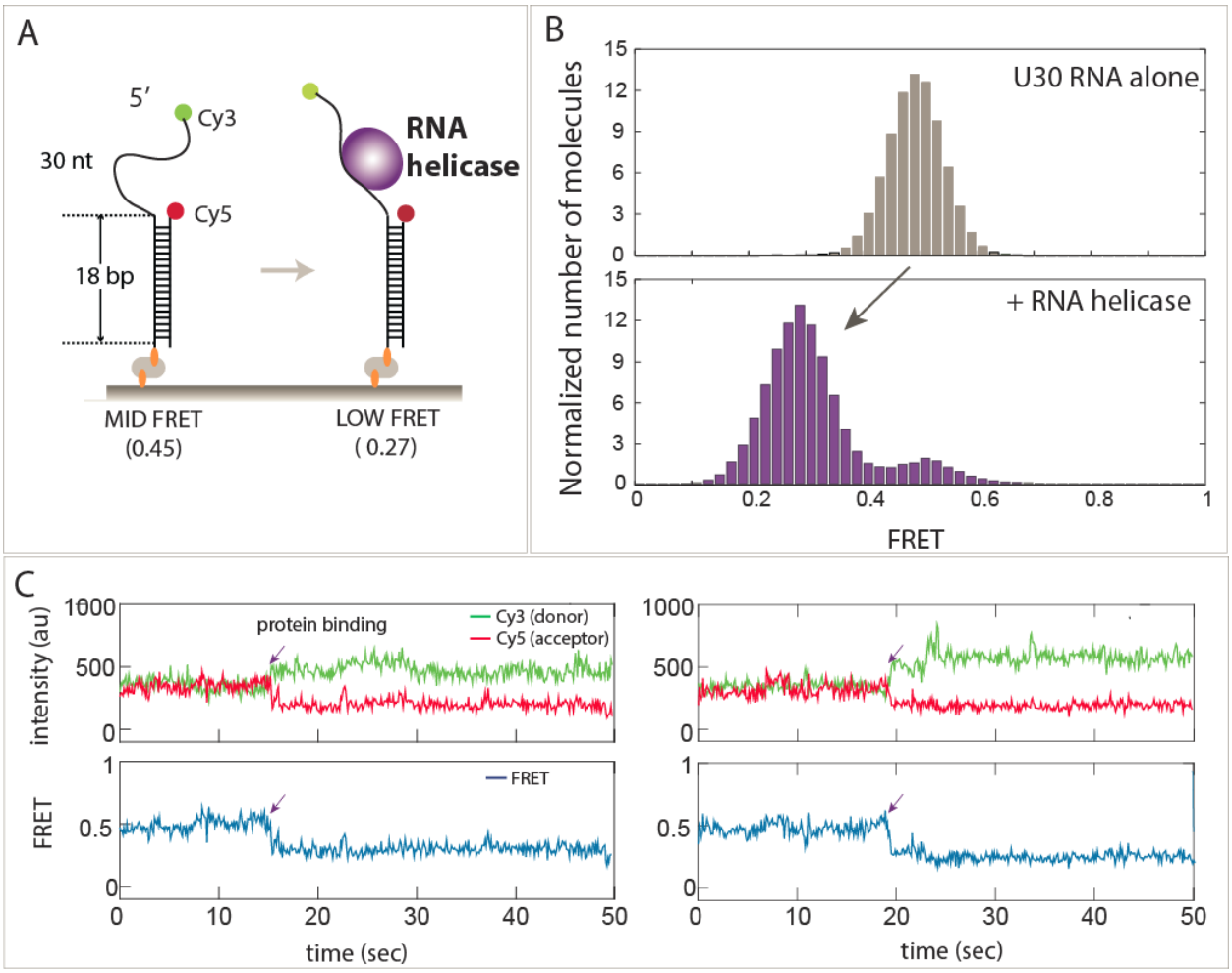

FIGURE S3 RNA helicase binding to U30 induces lower FRET, Related to Figure 2. (A) Schematic of protein binding to U30 RNA with FRET dyes. (B) FRET histogram before (gray, U30 alone) and after (purple, +RNA helicase) demonstrating that FRET became lower upon protein binding. (C) Representative single molecule FRET traces which show the FRET decrease induced by RNA helicase binding.

SUPPLEMENTAL FIGURE 4

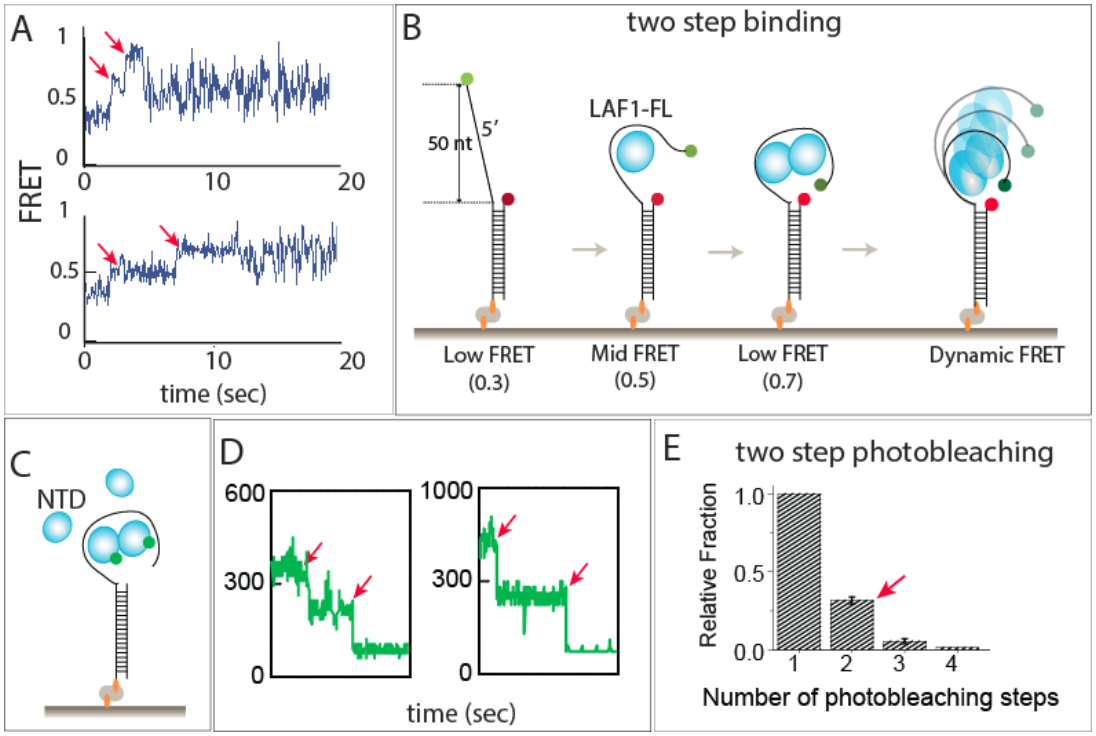

FIGURE S4 LAF-1-RNA stoichiometry, Related to Figure 3. (A) 10-15% of smFRET traces obtained in high LAF-1 concentration with U40 and U50 reveal two-step FRET increase. (B) Schematic diagram which indicates two LAF-1 molecules binding to ssRNA, giving rise to the dynamic FRET. (C) LAF-1-NTD was singly labeled with Cy3 dye with labeling efficiency of 50%. (D) Single molecule traces displaying two step photobleaching of Cy3. (E) Distribution of one to four step photobleaching molecules obtained in the experiment The labeling efficiency of NTD was approximately 50%, which means that there is 25% chance (0.5x0.5=0.25) of observing two labeled proteins binding RNA together. Our analysis indicates that there is indeed approximately 25% molecules that exhibit two step photobleaching, indicating 2:1 stoichiometry between LAF-1 and RNA.

SUPPLEMENTAL FIGURE 5

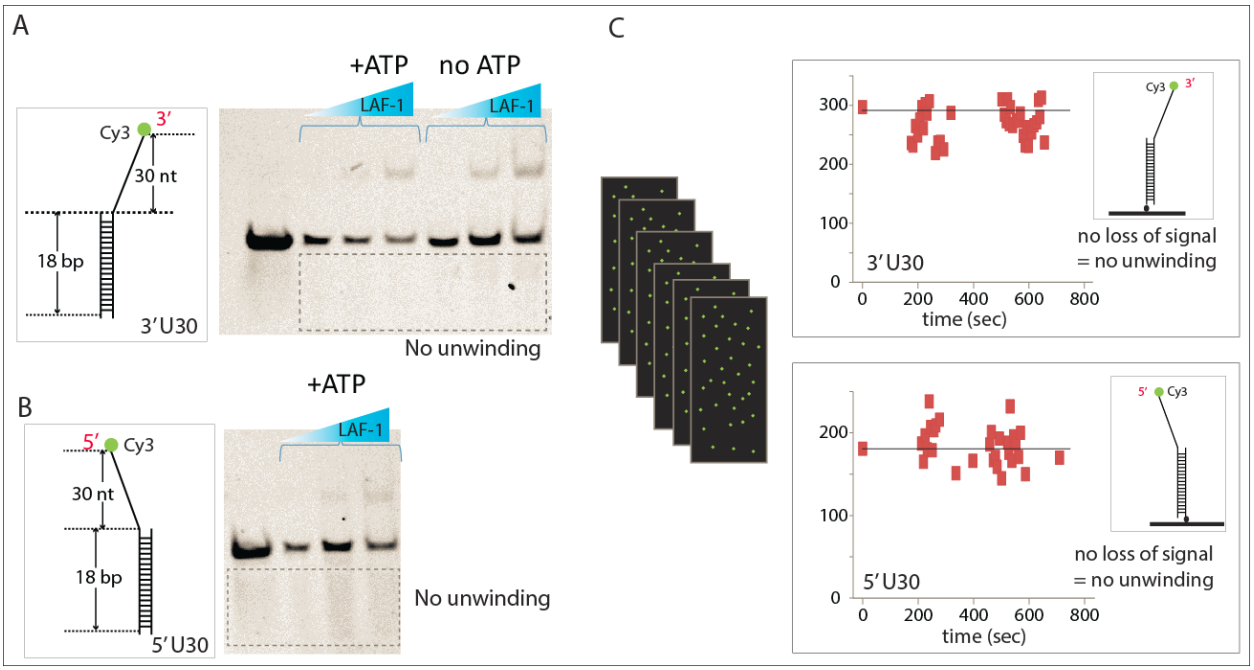

FIGURE S5 LAF-1 does not induce unwinding, Related to Figure 6. (A) LAF-1 applied to 3'U30 with and without ATP. EMSA gel shows no band that correspond to unwound strand. (B) LAF-1 applied to 5'U30 produced no unwound product with ATP. (C) Single molecule experiments were performed on both 3'30 and 5'U30 substrates in which complete unwinding of 18 base pairs will result in loss of Cy3 strand over time. Loss of signal over 12 minutes after the addition of LAF-1 and ATP indicate that there is no unwinding.

SUPPLEMENTAL FIGURE 6

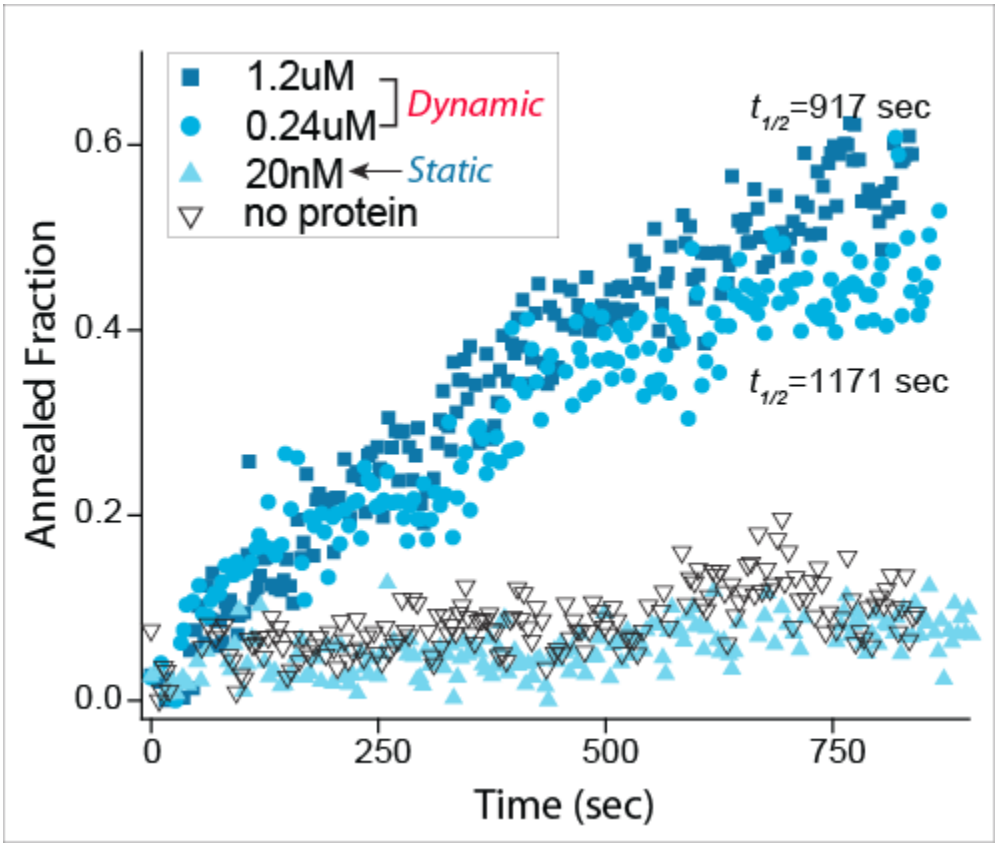

FIGURE S6 LAF-1 LAF-1FL displays concentration dependent RNA annealing, Related to Figure 6. Annealing experiment performed at 20nM LAF-1 displays negligible annealing whereas the same experiment done at 240nM and 1.2 μM exhibit drastically improved and accelerated annealing reaction. In agreement with the data shown in Figure 6F, high LAF-1 concentration, the condition that favors dynamic protein-RNA interaction stimulates RNA annealing, likely due to enhanced RNP-RNP interaction.

SUPPLEMENTAL FIGURE 7

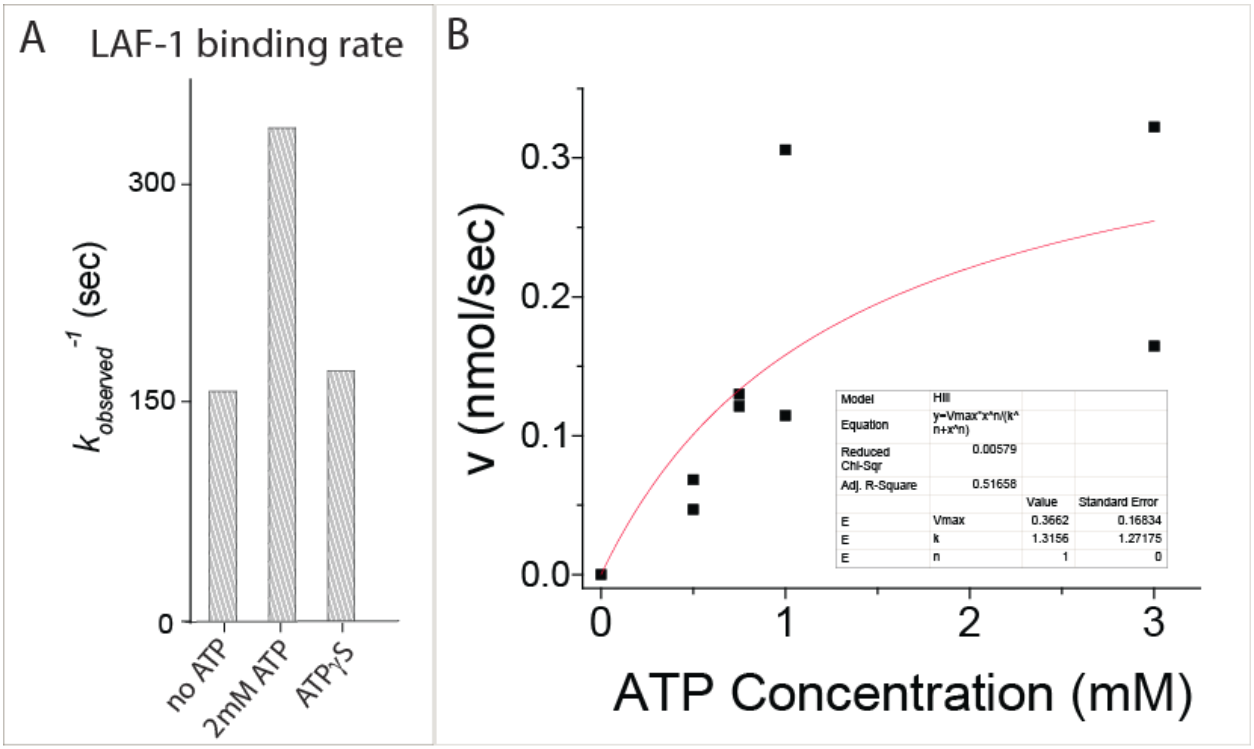

FIGURE S7 ATP hydrolysis rate and ATPγS effect, Related to Figure 7. (A) LAF-1 binding rate in no ATP, 2mM ATP and ATPγS conditions indicate that the ATP induced LAF-1 binding is due to ATP hydrolysis, not ATP binding. (B) Using Enzcheck Phosphate Assay Kit (Thermo Fisher, Catalot E6646), the ATP hydrolysis rate was measured. The result fitted to Michaelis-Menten equation reveals V_{max} = 0.3662 nmol/sec and K_m = 1.3156 mM.

TABLE S1

name	sequence
5´U15-50 (Fig 2-5)	5´- biotin-rArCrCrGrCrUrGrCrCrGrUrCrGrCrUrCrCrG-Cy5-3´ (up) 3´- rUrGrGrCrGrArCrGrGrCrArGrCrGrArGrGrC(rU)$_{15,\ 30,\ 40,\ 50}$-**Cy3** -5´ (down)
Annealing construct (Fig 6)	5´- biotin-rUrGrGrCrGrArCrGrGrCrArGrCrGrArGrGrC**Cy5** 3´- rArCrCrGrCrUrGrCrCrGrUrCrGrCrUrCrCrGrArGrArGrGrGrGrUrGrGrUrG rGrUrArGrUrGrArArCrCrArGrC-**Cy3**-5´ (down) 5'- rUrCrUrCrCrCrCrArCrCrArCrCrArUrCrArCrUrUrGrGrUrCrG(rA)$_{15}$-3´ (annealed)
40 bp RNA used in Fig S2	5'-rArCrGrCrUrUrArArCrArArCrCrArGrArUrCrArArArGAAArArArArCrArGrArCrArUrUrGrUrCrA-biotin-3' (up) 5'-rUrGrArCrArArUrGrUrCrUrGrUrUrUrUrUrUrCrUrUrUrGrArUrCrUrGrGrUrUrGrUrUrArArGrCrGrU-**DY547**-3' (down)

RNA sequences used in the study, Related to Figure 2-7.